AD_____

Award Number: DAMD17-99-2-9033

TITLE: In-Vitro Cytotoxicity Screening of Plant Extracts

PRINCIPAL INVESTIGATOR: Louis R. Barrows, Ph.D.

CONTRACTING ORGANIZATION: University of Utah
Salt Lake City, Utah 84102

REPORT DATE: June 2000

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION P

OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	June 2000	Annual (15 May 1999 - 14 May 2000)

4. TITLE AND SUBTITLE
In-Vitro Cytotoxicity Screening of Plant Extracts

5. FUNDING NUMBERS
DAMD17-99-2-9033

6. AUTHOR(S)
Louis R. Barrows, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Utah
Salt Lake City, Utah 84102

E-MAIL:
lbarrows@deans.pharm.utah.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; Distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT ***(Maximum 200 Words)***

In the current year of activity 78 plant extracts from Nigeria and Cameroon were screened for cytotoxicity in human cancer cell lines. These extracts were from plants used traditionally for antiparasitic activity, and a surprisingly high percentage (85%) were appreciably cytotoxic toward the human cancer cells. Active extracts were screened further for evidence of anticancer utility. These tests assessed mechanism of action by contrasting cytotoxicity in normal and tumor supressor gene deficient cell lines, and by assessing extract effects on cellular progression through the cell cycle. Of the extracts tested (19) exhibited activity indicative of DNA strand break activity. None exhibited activity indicative of covalent modification of DNA. Several extracts (11/14) had specific effects on the cell cycle, most causing the accumulation of cells in G2 or G1. Some had very unique effects on cell cycle. The active extracts are being fractionated and the active components are being described fully *in vitro*.

14. SUBJECT TERMS	15. NUMBER OF PAGES
	12
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

___ Where copyrighted material is quoted, permission has been obtained to use such material.

___ Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

___ Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

N/A In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Resources, national Research Council (NIH Publication No. 86-23, Revised 1985).

N/A For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

N/A In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

N/A In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

N/A In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

PI - Signature Date

TABLE OF CONTENTS

INTRODUCTION:

AP1 of the ICBG "Drug Development and Conservation of Biodiversity in West and Central Africa," provides data on the variety, distribution, abundance and dynamics of West and Central African flora. AP 2 focuses on determination of the chemical constituents of important West African plants. APs 3-5 are focused on the traditional uses and economic benefits derived from the flora. The research conducted under Program Project 6 is primarily concerned with development of African medicinal flora for their non-antiparasitic activities, one being anticancer activity. Screening extracts from traditionally used plants can capitalize on traditional knowledge in order to provide information about the potential alternative uses of known plants. Random screening of West African Flora for anticancer activity can provide information on the utility of species not recognized by traditional healers.

BODY:

As described in our approved statement of work our approach is to prioritize cytotoxic extracts based on screens which can detect cancer relevant mechanisms of tumor cell killing. We first test extracts for the ability to kill human cancer cells in culture. We then utilize mammalian cell based screens to detect molecules in the extracts that interfere with DNA metabolism or the cell cycle. In the mechanism screens cytotoxic extracts/molecules are tested for enhanced cytotoxicity in mutant cell lines which lack various cancer predisposition/tumor suppressor gene functions (caretaker functions). Cytotoxic extracts/molecules showing no DNA directed activity are tested by flow cytometry for the ability to disturb progression of normal cells through the cell cycle (tumor suppressor gatekeeper functions).

Extracts or molecules active in the mechanism-based screens are then examined for molecular mechanism of action in purified enzyme systems, and may be tested for anticancer activity in nude mouse human tumor xenograft models.

Our rationale for this approach is as follows: One feature common to most human cancers is genetic instability. Several cancer predisposition genes have DNA repair function, other tumor suppressor genes regulate cell cycle. In addition, most of the non-hormonal chemotherapeutics in the clinic damage DNA directly or interfere with DNA metabolism or disjunction. We have found that cytotoxicity serves as a good first screen because it usually reduces the number of extracts to be analyzed in depth approximately10 fold. The screens described below quickly identify extracts containing molecules with key anticancer activities. Furthermore, use of mammalian cell based screens allow rapid advancement to animal studies.

Comparison among four "isogenic" CHO lines identifies compounds that damage DNA or interfere with DNA metabolism and provides insight as to the molecular mechanism of action. Lead compounds are selected based on their interruption of the cell cycle or on

their enhanced toxicity toward a particular DNA repair deficient cell line. The cell lines used in the mechanism screen are described in Table I.

TABLE I

Cell Lines*

AA8**	CHO parental line	Thompson et al., Somat. Cell Genet. 6:391 (1980)
xrs-6	Ku 80 deficient, DNA PK & recombination deficient	Jeggo et al., Cancer Res. 49:7057 (1989)
EM9	XRCC1 & DNA ligase activity deficient, genetically unstable	Thompson et al., Somat. Cell Genet. 6:391 (1980)
UV20	ERCC1 deficient, DNA excision repair deficient, XP homologue	Thompson et al., Somat. Cell Genet. 6:391 (1980)

*All CHO lines adapted to microtiter culture, MTT colorimetric assay
**Cell line of choice for flow cytometry

In the current year of activity 78 plant extracts from Nigeria and Cameroon were screened for cytotoxicity in human cancer cell lines. These extracts were from plants used traditionally for antiparasitic activity, and a surprisingly high 85% were appreciably cytotoxic toward the human cancer cells. Active extracts were screened further for evidence of anticancer utility. Of the extracts tested 19, or 23%, exhibited activity indicative of DNA strand break activity. Experience has shown that the differential cytotoxicity profiles suggest specific mechanisms of DNA damage. Differentials in xrs-6 cells are indicative of direct chemical double strand scission, or topoisomerase II poisoning. Differentials in EM9 cells are indicative of single strand scission (usually via reactive oxygen species), or topoisomerase I poisoning. None of the extracts tested exhibited enhanced activity indicative of covalent DNA modification. Extracts exhibiting these activities (e.g., 848 - see spreadsheet in appendix) are currently being tested in purified DNA and human topoisomerase assays *in vitro*.

Several extracts were identified with specific effects on the cell cycle. The most striking effect is that of extract 799, which causes aneuploidy in CHO cells (Figure 1). The majority of these data were presented at the ICBG annual meeting in Douala Cameroon, February 2000. All testing results are presented in the spreadsheets in the appendix.

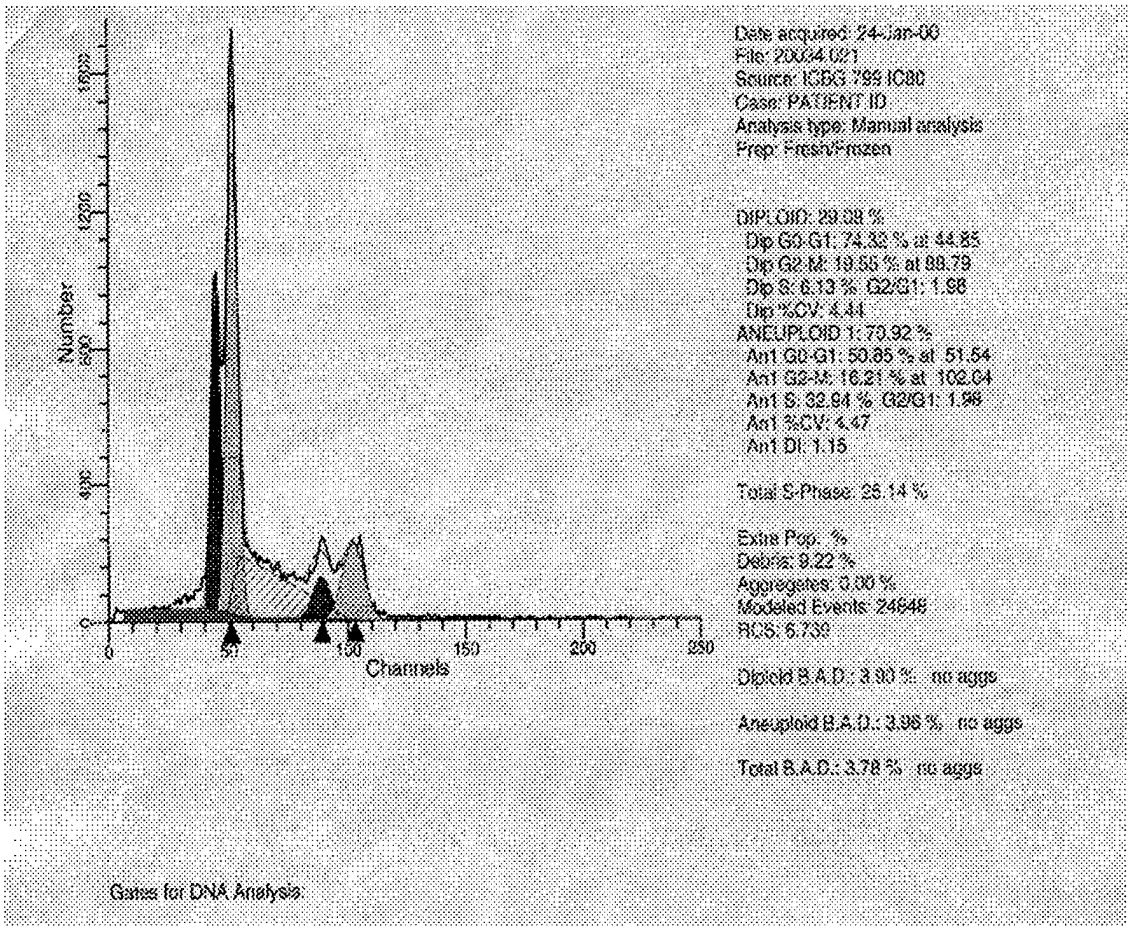

Figure 1.

KEY RESEARCH ACCOMPLISHMENTS:

- 78 extracts were screened for cytotoxicity.
- 67 (85 %) of the extracts were cytotoxic at 100 ug/ml or less.
- 19 (23 %) of the extracts appear to damage DNA or interfere with DNA metabolism.
- 11 of 14 extracts exhibited selective inhibition of cell progression through the cell cycle.

REPORTABLE OUTCOMES:

- The majority of these data were presented at the ICBG annual meeting in Douala Cameroon, February 2000, and were also included in the ICBG annual progress report. Manuscripts summarizing these data are in preparation.
- A Fogarty International Research Collaboration award was applied for, but not funded.
- This research has supplied rotation projects and contributed to the training of two graduate (Ph.D.) students at the University of Utah.

CONCLUSIONS:

Traditional medicines have been the source of several very important anticancer medicines (e.g., the vinca alkaloids, the podophyllotoxins and the camptothecins). The work in progress here has identified several medicinal plants, used traditionally in West Africa, as containing constituents with significant anticancer potential. Significant progress has been made towards identification of the molecular mechanism of these lead compounds. These extracts are being fractionated and the active components isolated via chromatography and bioactivity guided isolation. These molecules possess the potential to combat some of the most serious diseases confronting mankind today, and as such possess significant economic value if that potential is realized.

RFERENCES:

1) Thompson et al., Somat. Cell Genet. 6:391 (1980)
2) Jeggo et al., Cancer Res. 49:7057 (1989)

APPENDIX: (See Attached Spread Sheet of Testing Results)

NT = Not tested

✔ = Positive result

+/- = Borderline positive result

- = Negative result

ID#	Genus & species	Cytotoxicity 10µg/mL	Cytotoxicity 100µg/mL	XRS differential	XRS differential Second screen	Second/Third screen XRS	UV 20	EM9 differential	ID#
367		-	+	✓	-		-	✓	367
369		-	+	NT			-	✓	369
370		-	-	-			-	-	370
659		-	+	-		-	-	-	659
675		+	+	-			-	-	675
679		+/-	+	✓	-	✓	-	-	679
707		+	+	-			-	✓	707
716		-	-	NT			-	NT	716
719		+	+	✓	-		-	-	719
724	*Araliopsis tabouensis*	-	+	✓	+ -	✓	-	-	724
739		-	+/-	-				✓	739
740		+	+	✓	-	✓		-	740
742		+	+	✓	-			-	742
766		+	+	✓	-			-	766
769		+	+	-				-	769
798		+/-	+	-		-		-	798
799		+	+	✓	-	+ -		-	799
846		+/-	+	✓	+ -	✓		-	846
847		+/-	+	-				✓	847
848		+/-	+	✓	✓	✓		✓	848
1085		-	+	✓	+ -			✓	1085
1779		-	-	NT				NT	1779
1780		-	-	NT				NT	1780
1781		-	-	NT				NT	1781
1782		-	+	-				-	1782
1784		+	+	-				?	1784
1785		+	+	-				?	1785
1786		+	+	✓	?			✓	1786
1787		-	+	-				-	1787
1788		-	-	NT				NT	1788
1789		-	+	-				-	1789
1790		-	+	-				-	1790
1791		-	+	-				-	1791
1792		-	+	✓	-			-	1792
1793		-	+/-	NT				NT	1793
1794		-	+	-				-	1794
1795		-	+	-				-	1795
1796		-	+	-				-	1796
1797		-	+	-				-	1797
1798		-	+	NT				-	1798
1799		+/-	+	-				-	1799
1800		-	-	NT				NT	1800
1801		-	-	NT				NT	1801
1804		-	+/-	NT				NT	1804

ID#	Genus & species	Cytotoxicity 10µg/mL	Cytotoxicity 100µg/mL	XRS differential			EM9 differential	ID#
1806		-	+	✓	-	-	-	1806
1807		+/-	+/-	-			NT	1807
1808		-	+	-			✓	1808
1809		-	-	NT			NT	1809
1810		-	+	-			-	1810
1811		-	+	-			-	1811
1812		-	+	-			✓	1812
1813		-	+	✓	-		-	1813
1814		-	+	✓	+ -	✓	-	1814
1815		-	-	NT			NT	1815
1816		-	+/-	NT			NT	1816
1817		-	+	✓	-	✓	-	1817
1818		-	-	NT			-	1818
1819		-	+	-			NT	1819
1820		-	+	✓	-	✓	?	1820
1821		+	+	-			?	1821
1823		-	-	NT			NT	1823
1835		+/-	+	-			-	1835
1836		-	+	-			-	1836
1837		-	+	✓	+ -		-	1837
1838		-	+	-			-	1838
1839		-	+	✓	-		-	1839
1840		-	+/-	NT			NT	1840
1841		-	+	-			-	1841
1842		+	+	-			-	1842
1843		-	+	-			NT	1843
1844		-	+	✓	-		NT	1844
1845		+/-	+	-			NT	1845
1846		-	+	✓	-		NT	1846
1903							✓	1903

ID#	Cell Cycle - control			Cell Cycle - IC50			Cell Cycle - IC80		
	G0-G1	G2-M	S	G0-G1	G2-M	S	G0-G1	G2-M	S
367									
369									
370									
659									
675									
679	54.33%	13.10%	32.58%	58.78%	6.54%	34.68%	79.41%	7.31%	13.27%
707									
716									
719									
724									
739	51.07%	15.15%	33.77%				70.64%	13.58%	15.78%
740	51.58%	10.11%	38.31%	53.03%	11.09%	35.87%	61.63%	9.73%	28.64%
742	54.30%	12.89%	32.81%	68.99%	16.77%	14.25%	53.64%	22.30%	24.06%
766									
769									
798	53.00%	13.59%	33.41%	72.58%	6.30%	21.13%	56.42%	5.88%	37.69%
799	38.47%	12.39%	49.13%	58.61%	16.61%	24.78%	61.45%	13.79%	24.76%
846	50.74%	12.01%	37.25%	50.41%	8.01%	41.57%	66.43%	8.62%	24.95%
847									
848									
1085									
1779									
1780									
1781									
1782	53.76%	8.18%	38.06%	70.40%	10.98%	18.62%	75.37%	9.77%	14.86%
1784	55.68%	6.64%	37.68%	50.60%	8.58%	40.82%	65.12%	11.37%	23.52%
1785									
1786									
1787									
1788									
1789									
1790									
1791									
1792									
1793									
1794									
1795									
1796									
1797									
1798									
1799									
1800									
1801									
1804									
1806									
1807									
1808									
1809									
1810									

ID#									
1811									
1812									
1813									
1814									
1815									
1816									
1817									
1818									
1819									
1820									
1821									
1823									
1835									
1836									
1837									
1838									
1839									
1840									
1841									
1842									
1843									
1844									
1845									
1846									
1889	81.26%	4.77%	13.97%	59.63%	15.80%	24.58%	75.70%	11.90%	12.40%
1890	81.26%	4.77%	13.97%	53.71%	21.08%	25.21%	49.52%	12.33%	38.15%
1891	63.93%	16.41%	19.66%	44.46%	22.18%	33.36%	68.50%	16.63%	14.87%
1893	63.93%	16.41%	19.66%	44.77%	21.56%	33.67%	56.75%	16.87%	26.38%
1903	63.93%	16.41%	19.66%	69.14%	17.58%	13.28%	62.98%	30.15%	6.86%